

We claim:

1. An isolated microbial cell comprising an Environmentally Limited Viability System, wherein the cell is viable in a permissive environment and non-viable or temporarily viable in a non-permissive environment, the system comprising

(a) an essential gene, wherein expression of the essential gene in the cell is essential to the viability of the cell, the essential gene is expressed when the cell is in the permissive environment and is not expressed or temporarily expressed when the cell is in the non-permissive environment; and/or

(b) a lethal gene, wherein expression of the lethal gene is lethal to the cell and the lethal gene is expressed when the cell is in the non-permissive environment but not when the cell is in the permissive environment,

wherein the essential gene is essential for metabolism, growth, cell wall integrity, or cell membrane integrity of the cell.

2. The cell of claim 1 wherein the cell grows in the permissive environment and dies in the non-permissive environment.

3. The cell of claim 1 wherein the permissive environment comprises an environment containing a nutrient required to maintain expression of the essential gene, prevent expression of the lethal gene, or both, and the non-permissive environment comprises an environment lacking the nutrient.

4. The cell of claim 1 wherein the permissive environment is inside a warm-blooded animal and the non-permissive environment is outside a warm-blooded animal.

5. The cell of claim 1 wherein the lethal gene is carried on an extrachromosomal vector.

6. The cell of claim 1 wherein the lethal gene is carried on a chromosome of the cell.

7. The cell of claim 6 wherein the essential gene comprises the *asd* gene operatively linked to *araC-P_{BAD}*.

8. The cell of claim 5 wherein the vector has two lethal genes.

9. The cell of claim 8 wherein the vector comprises pMEG-104.

10. The cell of claim 1 wherein the cell is a gram-negative bacterium.

11. The cell of claim 10 wherein the gram-negative bacterium is an enteric bacterium.

12. The cell of claim 11 wherein the genus of the enteric bacterium is selected from the group consisting of *Escherichia* and *Salmonella*.

13. The cell of claim 1 wherein the essential gene is carried on an extrachromosomal vector.
14. The cell of claim 13 wherein expression of the essential gene is regulated by an expression product of a regulatory gene on a chromosome of the cell.
15. The cell of claim 13 wherein expression of the essential gene is regulated by an expression product of a regulatory gene on an extrachromosomal vector.
16. The cell of claim 1 wherein the essential gene is carried on a chromosome of the cell.
17. The cell of claim 5 wherein the system further comprises a replication gene carried on a chromosome of the cell, the expression of which is required for replication of the vector, wherein the replication gene is expressed in the permissive environment and is not expressed in the non-permissive environment.
18. The cell of claim 17 wherein expression of the replication gene is regulated by an expression product of a regulatory gene.
19. The cell of claim 5 wherein regulation of the extrachromosomal vector is regulated by an expression product of a regulatory gene,
wherein the absence of a functional expression product of the regulatory gene derepresses expression of the replication gene and wherein the expression product is not expressed or is inactive only in the non-permissive environment.
20. The cell of claim 1 further comprising an expression gene wherein the expression gene encodes a desired expression product.
21. The cell of claim 1 for use as a vaccine, wherein the cell is temporarily viable when in the an animal, the essential gene is temporarily expressed when the cell is in the animal, and the lethal gene is expressed when the cell is in the animal only after the cell has been in the animal for a period of time, wherein the permissive environment comprises an environment containing a nutrient required to maintain expression of the essential gene and prevent expression of the lethal gene, and the non-permissive environment comprises an environment lacking the nutrient.
22. The cell of claim 21 wherein the nutrient is arabinose.
23. The cell of claim 31 further comprising an expression gene wherein the expression gene encodes a desired expression product.
24. The cell of claim 1 for use as a vaccine, wherein the cell is viable when in the an animal and non-viable when outside of the animal, the essential gene is expressed when the

cell is in the animal and is not expressed when the cell is outside of the animal, and the lethal gene is expressed when the cell is outside of the animal and is not expressed when the cell is in the animal, wherein the permissive environment comprises a temperature of about 37°C and the non-permissive environment comprises a temperature of less than about 30°C.

25. The cell of claim 1 wherein the cell is temporarily viable in the non-permissive environment.

26. The cell of claim 1 further comprising a transfer vector.

27. The cell of claim 1 wherein the lethal gene is a gene required for excision of a prophage, wherein the prophage is in the chromosome of the cell, and wherein excision of the prophage causes lysis of the cell.

28. The cell of claim 1 wherein the essential gene, the lethal gene, or both have engineered expression.

29. A method of making a cell strain with environmentally limited viability comprising stably introducing into a cell

(a) an essential gene, wherein expression of the essential_gene in the cell is essential to the viability of the cell, the essential gene is expressed when the cell is in the permissive environment and is not expressed or temporarily expressed when the cell is in the non-permissive environment; and/or

(b) a lethal gene, wherein expression of the lethal_gene is lethal to the cell and the lethal gene is expressed when the cell is in the non-permissive environment but not when the cell is in the permissive environment,

wherein the cell strain is viable in a permissive environment and non-viable or temporarily viable in a non-permissive environment, and wherein the essential gene is essential for metabolism, growth, cell wall integrity, or cell membrane integrity of the cell.

30. The method of claim 29 wherein the permissive environment comprises a temperature of about 37°C and the non-permissive environment comprises a temperature of less than about 30°C.

31. The method of claim 29 wherein the permissive environment is inside a warm-blooded animal and the non-permissive environment is outside a warm-blooded animal.

32. The method of claim 29 wherein the cell is temporarily viable in the non-permissive environment.

33. An isolated microbial cell comprising an Environmentally Limited Viability System, wherein the cell is viable in a permissive environment and non-viable in a non-permissive

environment, the system comprising an essential gene, wherein expression of the gene in the cell is essential to the viability of the cell, and wherein said essential gene is expressed when the cell is in the permissive environment and is not expressed when the cell is in the non-permissive environment, and wherein said essential gene is essential for metabolism, growth, cell wall integrity, or cell membrane integrity of the cell.

34. The cell of claim 33 wherein the permissive environment comprises a temperature of about 37°C and the non-permissive environment comprises a temperature of less than about 30°C.

35. The cell of claim 33 wherein the permissive environment is inside a warm-blooded animal and the non-permissive environment is outside a warm-blooded animal, wherein the cell is a member of the *Enterobacteriaceae*.

36. The cell of claim 33 wherein the essential gene is carried on an extrachromosomal vector.

37. The cell of claim 33 wherein the cell is a gram-negative bacterium.

38. The cell of claim 37 wherein the gram-negative bacterium is an enteric bacterium.

39. The cell of claim 38 wherein the genus of the enteric bacterium is selected from the group consisting of *Escherichia* and *Salmonella*.

40. The cell of claim 33 wherein expression of the essential gene is regulated by the expression product of a regulatory gene.

41. The cell of claim 40 wherein the expression product of the regulatory gene inhibits expression of the essential gene and is expressed or active only in the non-permissive environment.

42. The cell of claim 36 wherein the system further comprises a replication gene carried on a chromosome of the cell, the expression of which is required for replication of the vector, wherein the replication gene is expressed in the permissive environment and is not expressed in the non-permissive environment.

43. The cell of claim 33 further comprising an expression gene wherein the expression gene encodes a desired expression product.

44. The cell of claim 33 for use as a vaccine, wherein the cell is viable when in an animal and non-viable when outside of the animal, the essential gene is expressed when the cell is in the animal and is not expressed when the cell is outside of the animal, wherein the permissive environment comprises a temperature of about 37°C and the non-permissive environment comprises a temperature of less than about 30°C, wherein the cell is a member of *Enterobacteriaceae*.

45. The cell of claim 44 further comprising an expression gene wherein the expression gene encodes a desired expression product.

46. A method of making a cell strain with environmentally limited viability comprising stably introducing into a cell an essential gene, wherein expression of the gene in the cell is essential to the viability of the cell, and the essential gene is expressed when the cell is in the permissive environment and is not expressed when the cell is in the non-permissive environment, and wherein the essential gene is a copy of a native gene, wherein the cell strain is viable in a permissive environment and non-viable in a non-permissive environment, wherein the native gene is inactivated in the cell.

47. The method of claim 46 wherein the permissive environment comprises a temperature of about 37°C and the non-permissive environment comprises a temperature of less than about 30°C.

48. The method of claim 46 wherein the permissive environment is inside a warm-blooded animal and the non-permissive environment is outside a warm-blooded animal, wherein the cell is a member of *Enterobacteriaceae*.

49. A method of inducing immunoprotection in a warm-blooded animal comprising administering to the animal a vaccine comprising a microbial cell comprising an Environmentally Limited Viability System, wherein the cell is viable when in the animal and non-viable when outside of the animal, the system comprising

an essential gene, wherein expression of the gene in the cell is essential to the viability of the cell, the essential gene is expressed when the cell is in the animal and is not expressed when the cell is outside of the animal; and

wherein the essential gene corresponds to an inactivated native gene of the cell, wherein the cell is a member of the *Enterobacteriaceae*.

50. The method of claim 49 wherein the system further comprises an expression gene wherein the expression gene encodes an antigen.

51. The method of claim 50 wherein the antigen is selected from the group consisting of bacterial antigens, viral antigens, plant antigens, fungal antigens, insect antigens, and non-insect animal antigens.

52. The method of claim 49 wherein the essential gene is carried on an extrachromosomal vector, and wherein the system further comprises a replication gene carried on a chromosome of the cell, the expression of which is required for replication of the vector, wherein the replication gene is expressed when the cell is in the animal and is not expressed when the cell is outside of the animal, wherein the cell is a member of the *Enterobacteriaceae*.

53. The cell of claim 40 wherein the absence of a functional expression product of the regulatory gene derepresses expression of the essential gene and wherein the expression product is not expressed or is inactive only in the permissive environment.

54. The cell of claim 33 wherein the essential gene is a gene essential for metabolism or growth of the cell.

55. The cell of claim 33 wherein the essential is a gene essential for cell wall or cell membrane integrity.

56. The cell of claim 33 wherein the essential gene is a modification methylase gene, a gene required for nucleic acid replication, or a gene encoding an enzyme that catalyzes steps in the biosynthesis of DAP.

57. The method of claim 33 wherein the essential gene is an *asd* gene, a *dap* gene, a *dal* gene, a *ddl* gene, a *fab*, gene, or a *pls* gene.

58. An isolated microbial cell comprising an Environmentally Limited Viability System, wherein the cell is viable in a permissive environment and non-viable in a non-permissive environment, the system comprising an essential gene, wherein expression of the gene in the cell is essential to the viability of the cell, the essential gene is expressed when the cell is in the permissive environment and is not expressed when the cell is in the non-permissive environment, and wherein the essential gene is derived from a gene in the host microorganism.